In Silico Investigation of Some Glucose-Aspirin as COX Inhibitor

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Abstract

Monosaccharide derived glucose-aspirin (GA) can be prepared by conjugation between glucose and aspirin (ASA). The GA is reported to show higher analgesic and anti-inflammatory properties than ASA itself. In this perspective, six GAs which are composed of \(\beta\)-D-glucopyranose, ASA and acetyl groups are considered for the present investigations. The glucose unit in these GAs possess regular chair conformation with slightly lower dipole moments. Molecular orbitals indicated a higher HOMO-LUMO gap of the molecules. All GAs showed more prone to electrophilic interactions than aspirin. Overall, glucose-aspirin esters are found to have better non-steroidal anti-inflammatory properties than the original aspirin. These GAs are better inhibitors of cyclooxygenase-2 (COX2, 5f19) compared to cyclooxygenase-1 (COX1, 6y3c) indicating that these GAs are potential drug candidates for COX2 related inflammation. Additionally, aspirinyl group at C-6 or C-3 position of the glucopyranose unit is found more suitable for anti-inflammatory activities as compared to C-4 position.

Keywords: Anti-inflammatory drug, Cyclooxygenase (COX), Aspirin, Molecular docking, Sugar esters.

1. Introduction

A common non-steroidal anti-inflammatory drug (NSAID) named aspirin (acetylsalicylic acid; ASA) is popular as a safe pain reliever \([1]\). It has been used for more than one hundred years. ASA has been used as antipyretic, analgesic, anti-inflammatory, antirheumatic, and antithrombotic drug \([2-3]\). It is used under several conditions such as headaches, toothaches, common cold, thromboembolic pulmonary hypertension, Felty's Syndrome, Reiter's syndrome, muscle pain, and peripheral artery disease. Among various advantages the most important is its use as primary and secondary prevention of cardiovascular diseases \([4-5]\). In fact, ASA can prevent myocardial attack and stroke. It was reported that around 55% of the elder people (>65 years) in USA are taking ASA daily or every other day \([6]\). In 1971 the exact pharmacological action mechanism of aspirin’s was discussed which is mentioned as irreversible cyclooxygenase (COX) inhibition \([7]\). In addition, suppression of
prostaglandin production along with COX inhibition was mentioned [8]. Many studies revealed that its COX inhibition action is due to the transfer of the acetyl group to the COX enzyme [9]. In other words, ASA acts as an acetylating agent to the COX (attached covalently with Ser529 of COX) and hence inactivates COX [8-9]. All these studies added value to the aspirin’s proper use, measure, and evidence-based education.

However, ASA has several side effects due to its uneventful frequent use i.e. misuse. General adverse effects are vomiting, nausea, excess stomach acid secretion, heartburn, and intestine irritation [10]. All of these may trigger kidney and liver diseases, seasonal allergies, gastrointestinal upset, ulcers and bleeding [11-12]. These adverse effects (toxicity) led to a mild controversy about its use, dosage or need for further modifications. It was observed that the attachment of the glucose unit with aspirin produces a more water-soluble aspirin analogue called glucose-aspirin (GA) [13]. The GA was found more stable with significant analgesic and anti-inflammatory activities [13-14]. The sugar part i.e. glucose part generally contributes to increased water solubility [15-20], biodegradability [21-23] and bioactivities [24-29] of the attached remaining part(s). For example, the addition of monosaccharide units as in amphotericin and/or nystatin increases their bioactivity and reduces their side effects [30]. The esters of different monosaccharides were also found to possess higher antimicrobial functionality [31-36]. With sugar moiety, GA also exhibited significant anti-cancer activity under in vitro conditions [13].

Very little literature is available on glucose-aspirin (GA) although it’s a superior potentiality to the aspirin (ASA) [13,14,37]. In principle, quantum chemical predictions are able to describe interactions between the molecule and biological receptors. Thus, in the present study six glucose-aspirins (GAs) are considered for thermodynamic and orbital properties, PASS analysis and molecular docking with COX enzyme.

2. Materials and methods

2.1. Glucose-aspirins (GAs) as COX inhibitors

In the present study, six glucose aspirins (Figure 1) are used mainly for the cyclooxygenase inhibitory activity study.
As a sugar unit β-D-glucopyranose is used. With this glucose unit, aspirinoyl group is shown to attach at C-6 (1), C-4 (2) and C-3 (3). Some of these compounds were synthesized by Jacob et al. [13-14]. For insight study of acetyl group here, acetyl group is also separately considered at C-2 position (1a-3a) in addition to the aspirinoyl unit. For comparative study aspirin (ASA, 4) is also used.

2.2. DFT based optimization

Online Chemspider was used to collect proper β-D-glucose geometry [38-39]. The aspirin unit was then added to this β-D-glucose at C-6, C-4 or C-3 position in GaussView software [40] to get different GAs (1-3). In addition, the acetyl group was added to get 1a-3a. For optimization of the GAs DFT (density functional theory) theory was used maintaining B3LYP method (6-31G+ basis set) in absence of solvent. It should be noted that for the inclusion of polarizable function 6-31G+ basis set was used. Optimized structures 1-3 and 1a-3a were used for docking with COX enzyme.

2.3. Thermodynamic properties, molecular orbitals and chemical reactivity calculation

The optimized structures were used for several thermodynamic properties prediction employing GaussView and WebMD [23]. Molecular orbitals like HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) possess salient features for their inherent reactivity. As an example, the HOMO of a drug molecule is generally related to its electron releasing ability. Optimized structures were opened in GaussView software. Then values of HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) values were calculated and converted into electron Volt. These values were further used to calculate their chemical reactivity descriptors using literature equations [41-43]. For example, chemical potential ($\mu$) = -(I+A)/2, where I = ionization potential and A = electron affinity.
2.4. PASS predication of glucose-aspirins

To predict and compare the aspirin related activities such as non-steroidal anti-inflammatory, antipyretic and anti-inflammatory properties we have employed predication of activity spectrum for substances (PASS) which is freely available after registration [44-45].

2.5. Method for molecular docking

Initially, the standard structure of aspirin in the SDF form was taken from ChemSpider. This compound and optimized glucose-aspirins are used as a ligand for docking studies. COX protein was taken from free online software named RCSB protein data bank (COX1 PDB id: 6y3c, and COX2 PDB id: 5f19). As the crystal structures possess water and hetero atoms the proteins were subjected to dehydration and hetero atoms removal followed by saving the file in PDB format. In the next step, the pdb file was energetically minimized in another software called Swisspdb.

For molecular docking, we mainly used PyRx autodock vina where ligands (compounds) and proteins (COX) are loaded duly. After minimization of their energy, they are converted into the necessary pdbqt file format. Before starting docking, the box sizes are maximized for autodock process and docked complex was opened with DiscoveryStudio for further study. Additionally, different interactions like nonbonding and hydrogen-bonding interactions among different GAs (ligands) and different amino acid residues of receptor protein(s) and binding affinities of ligand-protease were noted in the kcal/mole unit.

3. Results and discussion

3.1. Optimized structures of glucose-aspirins (GAs)

As in many of our studies, we emphasized conformational structures of carbohydrate part(s) of the compound(s), which may be interesting for activity studies [46-48]. In the case of GAs, the β-D-glucopyranosyl unit was found to exist in regular chair form with suitable $^4\text{C}_1$ conformation (Figure 2).

Figure 2. Structures of glucose-aspirins after DFT optimization (H atoms are not shown).
3.2. Thermodynamic studies

For comparison with aspirin, several thermodynamic properties are calculated from their DFT optimized files [49]. Table 1 indicated that due to the larger molecular size of glucose-aspirins (GAs) their electronic energy, Gibb’s free energy (GFE), enthalpy and entropy are higher than aspirin. However, due to positional effects of aspirin part in GAs variable dipole moments are found for these GAs and thereby the position of aspirin group with glucose unit imposes variable polar nature of GAs. It has been reported that in many bioactive molecules there is a correlation between dipole moment and medicinal property [50]. Generally, a higher dipole moment is favourable for medicinal application as that compound is more soluble in a polar solvent. It is noticed from Table 1 that although 1a (5.91 Debye) and 2a (6.11 Debye) possess greater dipole moments 3a (3.56 Debye) has a lower dipole moment. The greater dipole moment also indicates their higher mp/bp and solubility.

Table 1. Thermodynamic related properties of glucose-aspirins.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Electronic energy (Hartree)</th>
<th>GFE (Hartree)</th>
<th>Enthalpy (Hartree)</th>
<th>Entropy (cal/mol-K)</th>
<th>Dipole moment (Debye)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1259.1082</td>
<td>-1258.8353</td>
<td>-1258.7559</td>
<td>167.075</td>
<td>3.3264</td>
</tr>
<tr>
<td>1a</td>
<td>-1411.7203</td>
<td>-1411.4164</td>
<td>-1411.3268</td>
<td>188.474</td>
<td>5.9081</td>
</tr>
<tr>
<td>2</td>
<td>-1259.0992</td>
<td>-1258.8256</td>
<td>-1258.7437</td>
<td>164.795</td>
<td>3.1867</td>
</tr>
<tr>
<td>2a</td>
<td>-1411.7097</td>
<td>-1411.4052</td>
<td>-1411.3168</td>
<td>186.121</td>
<td>6.1119</td>
</tr>
<tr>
<td>3</td>
<td>-1259.0970</td>
<td>-1258.8260</td>
<td>-1258.7454</td>
<td>169.435</td>
<td>4.2439</td>
</tr>
<tr>
<td>3a</td>
<td>-1411.7143</td>
<td>-1411.4094</td>
<td>-1411.3214</td>
<td>185.200</td>
<td>3.5588</td>
</tr>
<tr>
<td>4</td>
<td>-649.6853</td>
<td>-649.5469</td>
<td>-649.4926</td>
<td>114.258</td>
<td>6.2099</td>
</tr>
</tbody>
</table>

3.3. Molecular orbitals and MEP of glucose-aspirins

To rationalize COX inhibitory activities and related results orbital properties of all the compounds were assessed as tabulated in Table 2. Here, hardness (η) is calculated as (I-A)/2 and softness (S) is equal to 1/η. HOMO-LUMO gap (Δԑ) of glucose-aspirins (GAs) is seen as higher than aspirin. However, the ionization potential (I), and chemical potential (µ) of both GAs and aspirin are almost similar (Table 2). Electron affinity (A) of 1, 1a, 2, and 2a are lower than the 3, 3a and 4. The lower electron affinity indicates their better stability. Again, the hardness of all the GAs is higher than aspirin and softness is lower than aspirin (4).

Table 2. Orbitals of glucose-aspirins and related properties.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>ԑLUMO</th>
<th>ԑHOMO</th>
<th>Δԑ</th>
<th>I</th>
<th>A</th>
<th>µ</th>
<th>η</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.886</td>
<td>-7.216</td>
<td>5.330</td>
<td>7.216</td>
<td>1.886</td>
<td>-4.551</td>
<td>2.665</td>
<td>0.375</td>
</tr>
<tr>
<td>1a</td>
<td>-1.880</td>
<td>-7.189</td>
<td>5.309</td>
<td>7.189</td>
<td>1.880</td>
<td>-4.535</td>
<td>2.655</td>
<td>0.367</td>
</tr>
<tr>
<td>2</td>
<td>-1.885</td>
<td>-7.295</td>
<td>5.410</td>
<td>7.295</td>
<td>1.885</td>
<td>-4.590</td>
<td>2.705</td>
<td>0.370</td>
</tr>
<tr>
<td>2a</td>
<td>-1.928</td>
<td>-7.344</td>
<td>5.416</td>
<td>7.344</td>
<td>1.928</td>
<td>-4.636</td>
<td>2.708</td>
<td>0.370</td>
</tr>
<tr>
<td>3</td>
<td>-2.042</td>
<td>-7.333</td>
<td>5.291</td>
<td>7.333</td>
<td>2.042</td>
<td>-4.688</td>
<td>2.646</td>
<td>0.378</td>
</tr>
<tr>
<td>3a</td>
<td>-2.243</td>
<td>-7.437</td>
<td>5.194</td>
<td>7.437</td>
<td>2.243</td>
<td>-4.840</td>
<td>2.595</td>
<td>0.385</td>
</tr>
<tr>
<td>4</td>
<td>-2.201</td>
<td>-7.135</td>
<td>4.934</td>
<td>7.135</td>
<td>2.201</td>
<td>-4.668</td>
<td>2.467</td>
<td>0.405</td>
</tr>
</tbody>
</table>

LUMO, HOMO, I, A, µ, η and S are expressed in eV.

Map process by molecular electrostatic potential (MEP) recognize one molecule from another in terms of biological interaction sites, receptor drugs and substrates like enzymes. MEP of all the compounds is presented in Figure 3.
Figure 3. MEP of glucose-aspirins (1-3 and 1a-3a) and aspirin (4).

Generally, MEP is represented by the colour of the surface of the molecule depending on the potential values. MEP can be determined by diffraction technique in the laboratory and can be predicted by a computational method. It should be noted that the red colour represents the highest area for electrophilic reaction and the blue colour represent the highest area for nucleophilic interaction. The red zone for 1-3 and 1a-3a are comparable to the aspirin (4).

The higher red zone of GAs 2 and 2a indicate their higher electrophilic nature than aspirin i.e. 4-O-aspirinoylglucose possess better electrophilic nature than 6-O-aspirinoylglucose (1, 1a) and 3-O-aspirinoylglucose (3, 3a). Again, attachment of glucose unit with aspirin in any position decreases their nuceophilicity (Figure 3). It was reported that carbonyl oxygen atoms contribute red-coloured negative potential and carbonyl carbon and hydrogen atoms contribute blue coloured positive potential [51]. A similar observation is found in the present study (Figure 3).

3.4. PASS predicted NSAID, antipyretic and anti-inflammatory properties

Aspirin related three bioactivities as non-steroidal anti-inflammatory, antipyretic and anti-inflammatory properties are predicted using PASS free software. These predicted values are presented in Table 2. All the glucose-aspirins (GAs) (except 3a) possess greater Pa (0.60-0.69) than the aspirin (0.55) indicating better non-steroidal nature of the GAs. Again, without acetyl group at C-2 position (1a-3a) of the glucose unit reduces the non-steroidal nature of the GAs (1-3, Table 2). Similarly, antipyretic potentiality of GAs (1-3) was also reduced with the introduction of acetyl group at the same position (1a-3a). The antipyretic property of GAs is lower than that of the aspirin. The anti-inflammatory effects of 6-O-asperinoylglucose (1, Pa = 0.78) and 4-O-asperinoylglucose (2, Pa = 0.77) are seen to be superior to the traditional aspirin (4, Pa = 0.76). Addition of acetyl part at C-2 (1a-2a) reduces anti-inflammatory effects than non-acetate (1-2) except 3a. Overall, glucose-aspirin esters are found to have better non-steroidal anti-inflammatory properties than the original aspirin. Also,
these activities depend on the position of aspirin moiety in GAs and the order is found as C-6 > C-4 > C-3.

Table 2. NSAID and other activities predicted by PASS.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>NSAID agent</th>
<th>Antipyretic</th>
<th>Anti-inflammatory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pa</td>
<td>Pi</td>
<td>Pa</td>
</tr>
<tr>
<td>1</td>
<td>0.687</td>
<td>0.005</td>
<td>0.644</td>
</tr>
<tr>
<td>1a</td>
<td>0.565</td>
<td>0.009</td>
<td>0.544</td>
</tr>
<tr>
<td>2</td>
<td>0.682</td>
<td>0.005</td>
<td>0.718</td>
</tr>
<tr>
<td>2a</td>
<td>0.558</td>
<td>0.009</td>
<td>0.633</td>
</tr>
<tr>
<td>3</td>
<td>0.606</td>
<td>0.007</td>
<td>0.764</td>
</tr>
<tr>
<td>3a</td>
<td>0.474</td>
<td>0.014</td>
<td>0.644</td>
</tr>
<tr>
<td>4</td>
<td>0.550</td>
<td>0.010</td>
<td>0.932</td>
</tr>
</tbody>
</table>

NSAID = Non-steroidal anti-inflammatory

3.5. Molecular docking: Binding affinity with COX

As aspirin and glucose-aspirins (GAs) are reported to possess anti-inflammatory and antipyretic properties (like other NSAID) two related proteins are considered for molecular docking. These are cyclooxygenase-1 (COX1) and cyclooxygenase-2 (COX2). As presented in Table 3, the addition of aspirinyl group at C-6 (as in 1 and 1a) and C-3 (as in 3 and 3a) position of β-D-glucopyranose unit increased GA’s binding potentiality with both the COX1 and COX2, while the addition of aspirinyl group at C-4 (as in 2 and 2a) decreased binding score compared to GA 1. In addition, GAs (1, 3; -7.9 to -8.3 kcal/mol) and their acetyl esters (1a, 3a; -6.5 to -8.2 kcal/mol) showed better binding scores than that of the standard drug ibuprofen (-6.5 to -6.7 kcal/mol). The docking scores are very much higher than the highly prescribed drug aspirin (ASA, Table 3). The binding energies of 1a and 3a are nicely stabilized by different non-bonding interactions (Figure 4). Overall, the GAs and their acetyl esters are better active against COX2 (5f19) compared to COX1 (6y3c).

Table 3. Molecular docking score (binding energy) with COX1 and COX2.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>6y3c (kcal/mol)</th>
<th>5f19 (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-7.9</td>
<td>-8.3</td>
</tr>
<tr>
<td>1a</td>
<td>-8.0</td>
<td>-8.1</td>
</tr>
<tr>
<td>2</td>
<td>-6.3</td>
<td>-7.6</td>
</tr>
<tr>
<td>2a</td>
<td>-6.6</td>
<td>-7.7</td>
</tr>
<tr>
<td>3</td>
<td>-6.5</td>
<td>-7.9</td>
</tr>
<tr>
<td>3a</td>
<td>-8.2</td>
<td>-8.2</td>
</tr>
<tr>
<td>4</td>
<td>-5.3</td>
<td>-5.8</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>-6.7</td>
<td>-6.5</td>
</tr>
</tbody>
</table>

*For rigorous validation ibuprofen is used for docking in addition to aspirin.
Interestingly, more active both the compounds 1a and 3a have higher electronic energy compared to 1, 2 and 3 (Table 1). Also, they have higher Gibb’s free energy and enthalpy. The docking results of GAs are found to be in agreement with the PASS predicted results as shown in Table 2.

4. Conclusion

Numerous superior anti-inflammatory and anticancer activities led us to design and in silico study six glucose-aspirins (GAs) as a non-steroidal anti-inflammatory drug (NSAID). The DFT oriented study indicated that all the GAs have regular chair conformation in their structures. Thermodynamically, their dipole moments are predicted lower than that of standard drugs namely aspirin (ASA). Molecular docking studies with cyclooxygenase-1 (COX1) and cyclooxygenase-2 (COX2) indicated that attachment of aspirinyl group at C-6 or C-3 position of glucopyranose unit is more suitable for anti-inflammatory activities as compared to C-4 position. The study also indicated that these GAs are potential drug candidates for COX2 related inflammation as compared to ibuprofen or aspirin.

References


